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ART UNIT	PAPER NUMBER
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/669,976	ENGEL ET AL.	
	Examiner	Art Unit	
	Stephanie K. Mummert, Ph.D.	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 February 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-16* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-16* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment filed on February 12, 2007 is acknowledged and has been entered. Claims 1-4 and 10-16 have been amended.

Claims 1-16 are discussed in this Office action.

All of the amendments and arguments have been thoroughly reviewed and considered but are not found persuasive for the reasons discussed below. Any rejection not reiterated in this action has been withdrawn as being obviated by the amendment of the claims. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

This action is made NON-FINAL.

Previous Rejections

The rejection of claims 3-4, 9-11 and 13-15 under 35 U.S.C. 112, second paragraph as being vague and indefinite are withdrawn in view of Applicant's amendment to the claims.

Claim Interpretation

The term 'hot start DNA polymerase' was not explicitly defined within the specification. Instead, the term was broadly referred to as "the same effect was also observed with enzymes which are either temporarily chemically activated such as HotStarTaq (Qiagen GmbH) as well as with antibody-blocked enzymes (data not shown)." (p. 12, paragraph 152 of PgPub). Therefore,

the term is being interpreted as reading on an enzyme such as HotStarTaq in addition to enzymes which are complexed with anti-Taq polymerase enzymes as part of the amplification reaction or composition.

New Grounds of Rejection

Claim Rejections - 35 USC § 103

1. Claims 12-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,703,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) as applies to claims 1-11 and 16 and further in view of Reed et al. (US Patent 5,459,038; October 1995) and Demke et al. (Biotechniques, 1992, vol. 12, no. 3, p. 333-334). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

Regarding claims 12-15, while Backus teaches amplification in the presence of Dextran sulfate as inhibiting to amplification, Demke provides an explanation that while dextran sulfate is inhibitory to PCR amplification, Dextran does not inhibit amplification via PCR.

Demke does not provide explicit teaching that dextran provides an improvement to PCR amplification without the inclusion of a volume exclusion agent. Reed teaches amplification of samples in the presence of Dextran (col. 19, lines 9-26, where the inclusion of Dextran results in more efficient amplification).

With regard to claim 12, Reed teaches an embodiment of claim 5, characterized in that

the volume exclusion reagent is a dextran (col. 19, lines 9-26, where the inclusion of Dextran results in more efficient amplification).

With regard to claim 13, Reed teaches an embodiment of claim 12, characterized in that the dextran has a molecular weight in the range of 1000 to 2,000,000 (col. 16, lines 10-20, where the dextran is T500, which comprises a MW of 500,000).

With regard to claim 14, Reed teaches an embodiment of claim 12, characterized in that the dextran has a molecular weight in the range of 3000 to 500,000 (col. 16, lines 10-20, where the dextran is T500, which comprises a MW of 500,000).

With regard to claim 15, Reed teaches an embodiment of claim 12, characterized in that the dextran has a molecular weight in the range of 40,000 daltons to 60,000 daltons (col. 16, lines 10-20, where the dextran is T500, which comprises a MW of 500,000, see obviousness rejection below).

While Reed teaches a Dextran of molecular weight 500,000, Reed also teaches that dextran generally provides an improvement over PCR amplification reactions that are not conducted in the presence of dextran. Thus, an ordinary practitioner would have recognized that the results optimizable variables of time, concentration and product amount could be adjusted to maximize the desired results. As noted in *In re Aller*, 105 USPQ 233 at 235,

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the results were other than routine, that the products resulting from the optimization have any

unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Backus to incorporate the fluorescent hybridization probes and coamplification of a target sequence of comparable copy number the of Bustin, to arrive at the claimed invention with a reasonable expectation for success. As taught by Bustin, "The accepted method for minimizing these errors and correcting for sample-to-sample variation is to amplify, simultaneously with the target, a cellular RNA that serves as an internal reference against which other RNA values can be normalized. The ideal internal standard should be expressed at a constant level among different tissues of an organism, at all stages of development, and should be unaffected by the experimental treatment. In addition, an exogenous control should also be expressed at roughly the same level as the RNA under study" (p. 182, col. 1). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to extend the coamplification in the presence of a polymeric volume exclusion agent taught by Backus to incorporate the teachings of Bustin with a reasonable expectation for success in order to achieve quantitative coamplification of two nucleic acid targets of similar copy number.

Furthermore, it would have been prima facie obvious in view of the teachings of Demke and Reed to include dextran into the method of amplification taught by Backus in view of Bustin. First, it is noted that Backus teaches amplification in the presence of PEG and dextran sulfate. While Backus teaches that dextran sulfate is inhibitory to amplification, Demke teaches "the inhibitory nature of some polysaccharides with free acidic groups is further demonstrated by

contrasting dextran and dextran sulfate. Dextran (neutral) has no interfering effects at 500:1 ratio, wheras dextran sulfate was very inhibitory (Table 1). Therefore, considering the teachings of Demke, it would have been prima facie obvious to substitute the dextran sulfate taught by Backus for the equivalent dextran as taught by Demke. Furthermore, as taught by Reed, "the inclusion of polysaccharide dextran (or similar) results in three unique advantages: firstly, its inclusion results in more efficient amplification leading to markedly higher sensitivity and specificity" (col. 19, lines 9-26). Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have extended the method taught by Backus to include dextran as taught by Reed and Demke to achieve efficient amplification with higher sensitivity and specificity with a reasonable expectation for success.

Previous Grounds of Rejection

Claim Rejections - 35 USC § 103

2. Claims 1-11 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,703,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

With regard to claim 1, Backus teaches a method for the coamplification of two or more target nucleic acids having different sequence compositions, said method comprising at least 15 primary amplification cycles (col. 2, line 67 to col. 3, line 3), each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture comprising two or more target nucleic acids and a hot start DNA polymerase, or their primer extension products, at a first temperature, T1, for denaturation of the strands of the target nucleic acids or their primer extension products (col. 3, lines 4-8), wherein said hot start DNA polymerase is activated at T1 (col. 7, lines 11-19, where the inclusion of a polymerase and the inclusion of an antibody specific to the DNA polymerase which inhibits enzymatic activity below about 50°C and which is inactivated at higher temperatures), and

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T2 (col. 3, lines 9-12), and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T3, provided that when priming and primer extension product formation are carried out in the same step, T2 and T3 are the same (col. 3, lines 13-18),

wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 20 weight % of a nonionic polymeric volume exclusion agent (col. 3, lines 19-21, where the disclosed percentage of 4 weight % falls within the range(s) claimed), and

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle, detecting two or more of the primer extension products having different sequence composition as an indication of coamplification of the target nucleic acids (col. 3, lines 46-48).

With regard to claim 3, Backus teaches a method for the coamplification of two or more target nucleic acids having different sequence compositions, said method comprising at least 15

primary amplification cycles (col. 2, line 67 to col. 3, line 3), each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture comprising two or more target nucleic acids and a hot start DNA polymerase, or their primer extension products, at a first temperature, T1, for denaturation of the strands of the target nucleic acids or their primer extension products (col. 3, lines 4-8), wherein said hot start DNA polymerase is activated at T1 (col. 7, lines 11-19, where the inclusion of a polymerase and the inclusion of an antibody specific to the DNA polymerase which inhibits enzymatic activity below about 50°C and which is inactivated at higher temperatures), and

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T2 (col. 3, lines 9-12), and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T3, provided that when priming and primer extension product formation are carried out in the same step, T2 and T3 are the same (col. 3, lines 13-18),

wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 20 weight % of a nonionic polymeric volume exclusion agent (col. 3, lines 19-21, where the disclosed percentage of 4 weight % falls within the range(s) claimed), and

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle, detecting two or more of the primer extension products having different sequence composition as an indication of coamplification of the target nucleic acids (col. 3, lines 46-48).

With regard to claim 2 and 4, Backus teaches a method for the coamplification of two or more target nucleic acids having different sequence compositions, said method comprising at least 15 primary amplification cycles (col. 3, lines 22-26), each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture comprising two or more target nucleic acids and a hot start DNA polymerase, or their primer extension products, at a first temperature, T1, for denaturation of the strands of the target nucleic acids or their primer extension products (col. 3, lines 27-31), wherein said hot start DNA polymerase is activated at T1 (col. 7, lines 11-19, where the inclusion of a polymerase and the inclusion of an antibody specific to the DNA polymerase which inhibits enzymatic activity below about 50°C and which is inactivated at higher temperatures), and

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T2 (col. 3, lines 32-35), and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T3, provided that when priming and primer extension product formation are carried out in the same step, T2 and T3 are the same (col. 3, lines 36-41),

wherein the reaction mixture in at least one of the primary amplification cycles comprises from l to 20 weight (col. 3, lines 42-45, wherein the disclosed percentage of 4 weight % falls within the claimed range(s)), and a DNA polymerase or a hot start DNA polymerase (col. 7, lines 11-19, where the inclusion of a polymerase and the inclusion of an antibody specific to the DNA

polymerase which inhibits enzymatic activity below about 50°C and which is inactivated at higher temperatures), and

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle, detecting two or more of the primer extension products having different sequence composition as an indication of coamplification of the target nucleic acids (col. 3, lines 46-48).

With regard to claim 5, Backus teaches a method according to one of claims 1 - 4, characterized in that the volume exclusion agent is selected from the group consisting of a polyether, a reaction product of a sugar with epichlorohydrin, a polysaccharide, and a polyacrylate (col. 7, lines 36-41).

With regard to claim 6, Backus teaches a method according to claim 5, characterized in that the volume exclusion agent is selected from the group of polyethers of the general formula;

$$H\text{-}(\text{-}O\text{-}R\text{-})_n\text{-}H$$

wherein R is an alkylene bridge of 1 to 6 carbon atoms - branched or unbranched - and n is an integer of 15 to 1000 (col. 3, lines 42-48).

With regard to claim 7, Backus teaches an embodiment of claim 6, characterized in that R may represent l,2-ethylene, l,3-propylene, l,2-propylene, 2-hydroxy-l,3-propylene, 3-hydroxy-l,2-propylene, l,4-butylene, l,3-butylene, or l,2-hexylene (col. 7, lines 48-52).

With regard to claim 8, Backus teaches an embodiment of claim 6, characterized in that the polyether is poly(ethylene glycol) (col. 7, lines 53-56, where it is noted that a preferred R group is polyethylene glycol).

With regard to claim 9, Backus teaches an embodiment of claim 8, characterized in that

the polytethylene glycol) has a molecular weight in the range of 1000 daltons to 2,000,000 daltons (col. 7, lines 59-67, where it is noted that the preferred range is between about 1000 to about 20,000, wherein the term about represents +/-10% and wherein the preferred range of molecular weights falls within the range as claimed, presuming that it is intended for the claimed ranges to be measured in daltons; col. 15, lines 19-21, where PEG-8000, a polyethylene glycol with a molecular weight of 8000 daltons was used in the examples).

With regard to claim 10, Backus teaches an embodiment of claim 8, characterized in that the poly(ethylene glycol) has a molecular weight in the range of 3000 daltons to 500,000 daltons (col. 7, lines 59-67, where it is noted that the preferred range is between about 1000 to about 20,000, wherein the term about represents +/-10% and wherein the preferred range of molecular weights falls within the range as claimed, presuming that it is intended for the claimed ranges to be measured in daltons; col. 15, lines 19-21, where PEG-8000, a polyethylene glycol with a molecular weight of 8000 daltons was used in the examples).

With regard to claim 11, Backus teaches an embodiment of claim 8, characterized in that the poly(ethylene glycol) has a molecular weight of about 8000 daltons (col. 7, lines 59-67, where it is noted that the preferred range is between about 1000 to about 20,000, wherein the term about represents +/-10% and wherein the preferred range of molecular weights falls within the range as claimed, presuming that it is intended for the claimed ranges to be measured in Daltons; col. 15, lines 19-21, where PEG-8000, a polyethylene glycol with a molecular weight of 8000 daltons was used in the examples).

With regard to claim 16, Backus teaches an embodiment of claim 5, characterized in that the polyacrylate is selected from the group consisting of poly(hydroxyethyl acrylate) or poly(2,3-

dihydroxypropyl acrylate) (col. 8, lines 12-15).

Regarding claims 1-4, Backus does not explicitly teach that the two or more target nucleic acids are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold. Regarding claim 2-4, Backus does not teach that the reaction mixture comprises a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridization.

Bustin teaches an overview of the quantitation of mRNA using a variety of methods, including quantitative real-time RT-PCR, a method which incorporates a variety of means of detection, including hybridization probes (Abstract).

With regard to claims 1-4, Bustin teaches the coamplification of nucleic acids which are present at comparable copy numbers, wherein the maximum difference between the lowest and highest copy number is 10-fold (p. 182, col. 1, 2nd paragraph, where the normalization of quantification of a target nucleic acid is accomplished through co-amplification of an internal control target sequence, referred to as an endogenous control. It is also noted that the endogenous control should be expressed at roughly the same level as the RNA under study; see also p. 185, 'multiplex RT-PCR' heading, where multiple primer sets are used to amplify multiple specific targets simultaneously).

With regard to claims 2-4, Bustin discloses a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridization (Figure 3 and p. 174, where molecular beacon probes were described; Figure 4A-C right side and p. 177, where 'hybdrization probes' were described; Figure 5, p. 177, where TaqMan hydrolysis probes were described).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Backus to incorporate the fluorescent hybridization probes and coamplification of a target sequence of comparable copy number the of Bustin, to arrive at the claimed invention with a reasonable expectation for success. As taught by Bustin, "The accepted method for minimizing these errors and correcting for sample-to-sample variation is to amplify, simultaneously with the target, a cellular RNA that serves as an internal reference against which other RNA values can be normalized. The ideal internal standard should be expressed at a constant level among different tissues of an organism, at all stages of development, and should be unaffected by the experimental treatment. In addition, an exogenous control should also be expressed at roughly the same level as the RNA under study" (p. 182, col. 1). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to extend the coamplification in the presence of a polymeric volume exclusion agent taught by Backus to incorporate the teachings of Bustin with a reasonable expectation for success in order to achieve quantitative coamplification of two nucleic acid targets of similar copy number.

Response to Arguments

Applicant's arguments filed February 12, 2007 have been fully considered but they are not persuasive. Regarding the previous rejection of claims 1-14 and 16 under 35 U.S.C. 103 as being unpatentable over Backus in view of Bustin, Applicant traverses the rejection.

Regarding Backus, Applicant asserts "there is no teaching in Backus to combine a volume exclusion agent and hot start polymerase for the coamplification of two or more target nucleic acids present at comparable, i.e., less than 10-fold different, copy numbers as disclosed in the present specification" (p. 15 of remarks).

First, in response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Further, regarding Backus, Applicant asserts "the results presented in Backus actually teach away from the surprising results shown in the present application" and notes "As seen in Table II of Backus, the presence of a volume exclusion agent, i.e., dextran, totally inhibits the PCR amplification reaction. According to Backus, 'it is clear that... the presence of dextran sulfate totally inhibits PCR'" (p. 15-16 of remarks).

First, it is noted that the rejection in view of Backus as relates to the claims referring specifically to dextran have been modified in view of Applicant's arguments. However, as noted in the new rejection above, the inclusion of dextran sulfate is specifically inhibitory, while dextran alone is not and the prior art teaches that dextran provides an improvement in amplification. Therefore, the rejection is maintained with modifications.

Regarding Bustin, Applicant asserts "the section of Bustin referred to by the Examiner concerns the normalization, i.e., calibration, to account for errors that occur as a result of variations in the amount of mRNA starting material in mRNA reactions when quantifying mRNA transcripts again, particularly when comparing amplification of transcripts from samples

taken from different individuals". Applicant also states "Bustin relates to normalization of an RT-PCR reaction using an RNA with known in vitro expression levels. Bustin does not, as asserted by the Examiner, 'teach the coamplification of nucleic acids which are present at comparable copy numbers, wherein the maximum difference between the lowest and highest copy number is 10-fold" (p. 16-17 of remarks).

These areguments are not persuasive. While Applicant is correct that Bustin teaches calibration of RT-PCR reactions to account for errors that occur as a result of variations in the amount of starting mRNA, Applicant's characterization that the amplification of two targets simultaneously does not meet the limitation of co-amplification of nucleic acids which are present in comparable copy numbers is incorrect. As taught by Bustin, "the accepted method for minimizing these errors and correcting for sample-to-sample variation is to amplify, simultaneously with the target, a cellular RNA that serves as an internal reference against which other RNA values can be normalized" and also states "in addition, the endoegenous control should also be expressed at roughly the same level as the RNA under study" (p. 182), which meets the limitation of coamplification of more than one target that are present at comparable copy numbers. The rejection is maintained.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephanie K. Mummert, Ph.D. whose telephone number is 571-272-8503. The examiner can normally be reached on M-F, 9:00-5:30. If attempts to reach the

examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Stephanie K. Mummert

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